

## REMARKS

Claims 1, 2, 4, 7, 8, 19 and 21 are of record pending in this application. Claims 1 and 21 are hereby amended, and no claims are currently added or cancelled. Claims 1, 2, 4, 7, 8, 19 and 21 remain pending and in issue in the application.

Reconsideration of all outstanding rejections for all such claims remaining here in issue, i.e., claims 1, 2, 4, 7, 8, 19 and 21 and re-examination and allowance of all claims 1, 2, 4, 7, 8, 19 and 21 are hereby respectfully requested. After a short discussion regarding the Examiner Interview, the issues of the outstanding Office Action, mailed March 26, 2010 (hereafter, "the Office Action"), will be addressed *seriatim*.

### The Interview and Interview Summary

Applicants note with thanks that the Examiner held a telephonic interview with the undersigned attorney-of-record on 6 April 2010. Moreover, Applicants note that the Examiner mailed a Summary of that Interview on 12 April 2010, and that Applicants generally agree with the contents of that Summary and do not here dispute the accuracy of this Summary.

Moreover, Applicants respectfully submit that the Examiner's Summary of the Interview included all the minimum elements of and details necessary to make this interview sufficiently of-record in this case. If, for whatever reason, this indeed not so, Applicants respectfully request being provided notice of what is purportedly lacking, and being provided a minimum time period for providing the necessary material in response.

### Claim Objection

Claim 21 stands objected to due to the informality of an errant hyphen. This Claim 21 has been amended, the hyphen being deleted and an underlined space added between the two words,

“an” and “enzyme” in line 2. The underlineation is added merely to indicate addition of the space and is not intended to appear as such in the final form of the claim. Applicants respectfully submit claim 21 is in proper form so the objection can be withdrawn. Action to this end is respectfully requested.

### **Claim Rejections under 35 USC §103(a)**

Claims 1, 2, 4, 7, 8, 19 and 21 were rejected under 35 USC § 103(a) as purportedly being unpatentable over Mochs et al. (The Plant J. 11(2): 227-236, 1997; hereinafter “Mochs”) in view of Day et al. (FEBS letters 486 (1998); hereinafter “Day”), Arend et al. (Biotechnol. Bioeng. 76(2):126-31, 2001, esp. pp. 129-130; hereinafter “Arend”) and Priefert (Applied Microbiol. Biotechnol. 56:296-314 (2001); hereinafter “Priefert”). Applicants respectfully traverse these rejections for at least the reasons discussed below, and respectfully submit that Applicants’ developments would indeed not be obvious to one skilled in the art.

Applicants reincorporate here in full the remarks presented in the Office Action Responses of August 5, 2009 and March 5, 2010, including the Declarations of Birger Møller and Jorgen Hansen, and respectfully submit that none of the cited references form a proper basis, whether taken alone or in any combination, for rejection under 35 USC §103(a).

Applicants’ present specification and claims provide detailed technical information on the introduction of the complete and discrete biosynthesis pathways of both vanillin and a glycosyltransferase into a single yeast cell and for the consequent formation of both the vanillin and the glycosyltransferase in the cell, which in turn interact to create the glycosylated vanillin in the cell as well. This provides for increased production of the vanillin aglycon, whether in glycosylated and/or non-glycosylated forms, or both. The glycosylation in the cell means a lesser quantity of the pure aglycon appears in the cell, and thus allows for the aglycon to appear as less toxic to the cell. The cell then produces still more aglycon. This additional production is more than it would have produced without the glycosylation in the cell. The aglycon in both

forms is then expressed and collected. The glycosylated form is then deglycosylated and the overall quantity produced is much more than would expected from the teachings of the art.

In Moebs, a pathway for production of a glucosyltransferase (SGT) was introduced into the yeast cell, but NOT a biosynthesis pathway for the aglycon compound solanidine. Indeed, production of the aglycon is not a part of the Moebs disclosure. Rather, the aglycon is manufactured elsewhere and a fixed amount introduced into the medium in which the yeast is disposed. The goal of Moebs is not production of aglycon nor of glycosylated aglycon as a product to-be-collected; though the glycosylated form is a desired by-product only inasmuch as this creates a less toxified environment for yeast growth. I.e., creation of the glycosylated form in Moebs is to de-toxify the environment for the goal of increased yeast growth, not for the purpose of creating a glycosylated aglycon product, and moreover not to increase aglycon formation. The aglycon production is not affected by the Moebs SGT alteration of the yeast there. The aglycon quantity is un-changed.

Moreover, the glycosylation that does occur, occurs outside the yeast cell in Moebs, and thus suggests nothing of how or what would occur if this glycosylation occurred in the cell, where by the current disclosure we find that it leads to increased aglycon production in and by the cell. And, again; indeed due to the fixed amount of aglycon present in the Moebs medium, there is no increased quantity formation of glycosylated aglycon. Glycosylated or not, there is no increased production of aglycon, the amount remains fixed.

As to the issue of the present results and the unexpectedness thereof, please see for example, paragraph [0294] of the present application, the publication version, US2006/0275877A1, in working example 15 which reads:

The conclusion is that vanillin glucoside can be formed in vivo in *Saccharomyces cerevisiae* by the glucosylation of vanillin. As vanillin glucoside is much less toxic to microbial organisms than is the aglycon vanillin, we conclude that expression of appropriate UDP-glucose glucosyltransferases in *Saccharomyces* yeast allows for overproduction of vanillin.

This comes from the recognition in paragraph [0200] that:

Regarding toxicity is show in examples 1 and 2 that the presence of a heterologous UDPG-glycosyltransferase in a microorganism [e.g. yeast cells] can increase its tolerance to vanillin through glucosylation.

Further, please see paragraph [0020] where:

The inventors found that *the microorganism [e.g. yeast]* with the glycosyltransferase during culture fermentation is capable of producing *higher [in vivo] amounts of the glycosylated form of the aglycon [e.g. glycosylated vanillin] as compared to the amounts of the corresponding aglycon [e.g. vanillin as such]* produced by the microorganism without the glycosyltransferase. See working examples herein for an illustrative examples ... (emphasis added).

In explanation, included with our RCE dated March 5, 2010, inter alia, was the declaration of inventor Joergen Hansen, itself dated February 28, 2010. In and attached to this declaration, as Exhibit A, was the article, de Novo Biosynthesis of Vanillin in Fission Yeast (*Schizosaccharomyces pombe*) and Baker's Yeast (*Saccharomyces cerevisiae*) where Joergen Hansen is one of the authors, published in the journal APPLIED AND ENVIRONMENTAL MICROBIOLOGY (see declaration of Hansen, paragraph 14, et al. and Exhibit A thereto). This article was discussed in, e.g., paragraphs 14-16 of the Joergen Hansen declaration. This 2009 article describes later made work, which was based on and followed the technical disclosure of the currently in issue application, 10/561,823, published as US2006/0275877A1.

Of relevance herein is e.g. Figure 4 on page 2271 – copied below (arrow and dashed line emphasis added).

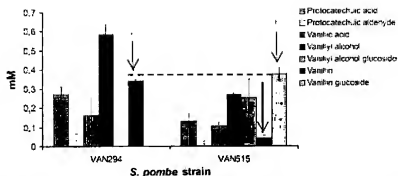


FIG. 4. Accumulation of vanillin, vanillin catabolites, intermediates, and glucosides in vanillin-producing *S. pombe* strain VAN294 alone or with co-expression of UGT72E2 strain VAN515. The numbers are averages of three experiments.

The VAN515 *S. pompe* yeast cell is a within the scope of the presently in issue claim 1 – i.e. the yeast cell comprises a glycosyltransferase (GT).

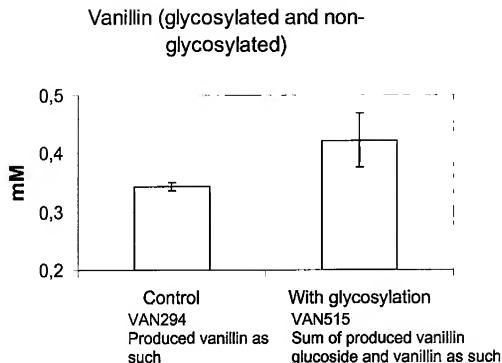
The VAN294 cell is a “control” cell herein – i.e. it is the same yeast cell without the GT.

As can be seen from Figure 4 – the VAN294 “control” cell does of course not make vanillin glucoside (i.e. the glycosylated aglycon), since it comprises no relevant glycosyltransferase (GT).

Of herein interest is that the vanillin glucoside column for the VAN515 cell (most to the right marked with arrow) is higher than the vanillin as such (the aglycon) column for the VAN294 “control” cell (black column marked with arrow).

Further, the VAN515 cell also produced some vanillin (aglycon in non-glycosylated form) as such (see VAN515 black column marked with arrow).

Below is given a figure, wherein the sum of VAN515 produced vanillin glucoside and vanillin as such of Figure 4 is shown to the right of the figure. To the left of the figure below is shown the amount of vanillin as such produced in the VAN294 “control” cell.



As can be seen in the figure above - it is clear that the data of the article confirms the technical description of the present application, 10/561,823 (publication US2006/0275877A1) – i.e. **these Figure 4 data confirms that by introducing a glycosyltransferase (GT) into the VAN294 “control” yeast cell one obtained a VAN515 yeast cell that produces significant higher amount of vanillin glucoside as compared to the amount of vanillin as such produced in the VAN294 “control” cell.**

As discussed in the declaration of inventor Joergen Hansen –this scientific article provides additional data supporting the method and claims of the current 10/561,823 application.

Moreover, also from the Hansen and Moller declarations, the scientific community has provided *SECONDARY CONSIDERATIONS* (see Graham v. John Deere) in the form of the Nature and Science articles, providing peer acceptance and laudatory commentary, a number of herein relevant positive comments to the work described in the article of Joergen and others as discussed above.

For instance – as discussed the paragraph 16 of the Joergen declaration – the highly esteemed **Science news** (Exhibit C in declaration) reads (emphasis):

***“To further **increase the yeast yield of vanillin**, the researchers **added an additional gene** that encodes for an enzyme that converts the straight vanillin into a form with a sugar attached, **vanillin beta-D-glucoside**. **This form isn’t toxic**, says Moeller, **allowing the yeast to hold more of the compound**”*** (emphasis added).

In short, **one may here say that the well known prestigious journal Science here acknowledges the positive technical improvement of the method in accordance with the present claim 1 to make vanillin in high yields** via an “intermediate step”, where high yield of vanillin glycoside is first made in vivo and this vanillin glycoside is then deglycosylated to get the final vanillin as such product.

The Nature article similarly provides peer accolades sufficient to provide evidence of non-obviousness, and thus patentability.

The obviousness rejections are thus obviated/traverses and can be withdrawn. Applicant therefore respectfully requests reconsideration and withdrawal of all rejections and consequently re-examination and allowance of all claims pending in this application; namely claims 1, 2, 4, 7, 8, 19 and 21.

### CONCLUSION

Applicant respectfully requests that all of the claims be re-examined and allowed. A timely Notice of Allowance is requested to be issued in this case. Applicants believe that no additional fees or petitions are due with this filing. However, should any such fees or petitions be required, please consider this a request therefore and authorization to charge Deposit Account No. 02-2093 as necessary.

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Respectfully submitted,

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